to give a partial soln of finely divided Na_3PSO_3. DMF (10 ml) was added, and the mixt was allowed to warm to 25°. Powdered **12a** (7.68 g, 20.0 mmoles) was then added in portions. Complete soln occurred after 10–15 min. Stirring was contd, and after 30–40 min cryst **13b** began separating. EtOH (500 ml) was added; and, after overnight refrign, the solid was collected, washed with EtOH, and recrystd twice from H_2O (30 ml)–EtOH (300 ml). The collected product, washed successively with EtOH and Et_2O, was air-dried and then allowed to equilibrate at const 50% relative humidity.

S-**2-[3-(*cis*-1,2-Cyclohexanedicarboximido)propylamino]-ethyl sodium hydrogen phosphorothioate (13d)** was prepd from **12b** (15.0-mmole scale) as described above for the conversion of **12a** into **13b**. The product that pptd from the EtOH-dild reaction mixt was recrystd from refrigd H_2O (50 ml)–EtOH (50 ml) soln, air-dried, and equilibrated at 50% relative humidity.

S-**2-[4-(*cis*-1,2-Cyclohexanedicarboximido)butylamino]ethyl Sodium Hydrogen Phosphorothioate (13f).**—Adaptation of the procedure described for the prepn of **13d** readily afforded **13f**.

Acknowledgments.—The authors are indebted to Dr. D. P. Jacobus and Dr. T. R. Sweeney for antiradiation data and to Dr. W. C. Coburn, Jr., and members of the Molecular Spectroscopy Section of Southern Research Institute for spectral data.

Antistaphylococcal and Antifibrinolytic Activities of ω-Amino Acids and Their L-Histidine Dipeptides[1,2]

AKIRA FUJII, KINJI TANAKA, YOSHIKI TSUCHIYA, AND ELTON S. COOK*

Division of Chemistry and Biochemistry, St. Thomas Institute, Cincinnati, Ohio 45206

Received May 7, 1970

The relationship was investigated between the molecular structure and the antistaphylococcal and antifibrinolytic actions of ω-amino acids and their L-histidine dipeptides, of which δ-aminovaleryl-L-histidine (**9**) and ε-aminocaproyl-L-histidine (**10**) are newly synthesized. The antistaphylococcal properties were demonstrated through their protective effects against staphylococcal infections in mice. The antifibrinolytic activities were determined *in vitro* by measuring prolongation of lysis time of a fibrin clot. The order of antistaphylococcal potencies of these compounds was: (a) glycine (**1**) < β-alanine (**2**) < γ-aminobutyric acid (**3**) < ε-aminocaproic acid (**5**) < δ-aminovaleric acid (**4**) and (b) glycyl-L-histidine (**6**) < β-alanyl-L-histidine (**7**) < γ-aminobutyryl-L-histidine (**8**) < δ-aminovaleryl-L-histidine (**9**) < ε-aminocaproyl-L-histidine (**10**). Comparing a and b, the protective power of ω-aminoacyl-L-histidines was much higher than that of the corresponding ω-amino acids. The order of antifibrinolytic potencies of ω-amino acids was identical with that of antistaphylococcal action except that **4** < **5** in the former. Practically no antifibrinolytic activity of ω-aminoacyl-L-histidines was observed *in vitro* under the conditions we employed.

It was previously reported that, by a prophylactic procedure, homocarnosine (**8**) and carnosine (**7**) protected C3H/HeJ mice[3a,b] but only **8** protected Swiss albino mice[3b,c] from death by *Staphylococcus aureus* infections. In this work, a series of component ω-amino acids was also examined and compared with the peptides by a combined prophylactic–therapeutic procedure with Swiss albino mice.[4]

The compounds discussed in this paper are: ω-amino acids, $H_2N(CH_2)_nCOOH$, where $n = 1$, glycine (**1**); $n = 2$, β-alanine (**2**); $n = 3$, γ-aminobutyric acid (**3**); $n = 4$, δ-aminovaleric acid (**4**); $n = 5$, ε-aminocaproic acid (**5**); and ω-aminoacyl-L-histidines, $H_2N(CH_2)_nCO$-His, where $n = 1$, glycyl-L-histidine (**6**); $n = 2$, β-alanyl-L-histidine (**7**) (carnosine); $n = 3$, γ-aminobutyryl-L-histidine (**8**) (homocarnosine); $n = 4$, δ-aminovaleryl-L-histidine (**9**); $n = 5$, ε-aminocaproyl-L-histidine (**10**).

The results indicate that **4** and **5**, higher homologs of **3**, were more effective against staphylococcal infections than **3**. Comparing **8** with **3**, and also **7** with **2**, both histidine dipeptides were more potent than their component ω-amino acids. These two facts suggested to us that both **9** and **10** might have even higher activities than **8**, as verified by the data in Figure 1.

The mechanism of the antistaphylococcal action also has been considered because none of the ω-amino acids and ω-aminoacyl-L-histidines tested showed bactericidal or bacteriostatic effects *in vitro*. We have been especially interested in the possible relationship between antistaphylococcal and antifibrinolytic activities, since **4** and **5** were reported to have antifibrinolytic activity.[5]

Chemistry.—Two general synthetic procedures were used to prepare the ω-aminoacyl-L-histidines in this study. The first was the phthalyl method, a modification of the one described by Sheehan and Frank[6] and similar to that reported by Turner,[7] except for the final purification process, in which we used ion-exchange chromatography and the phenol–calcium hypochlorite color reaction for the isolation and detection of ω-amino acids and their histidine dipeptides. The carbobenzoxy method, used as the second procedure, was modified from Bergmann and Zervas,[8] Sifferd and duVigneaud,[9] and Pisano, *et al.*[10] The yields, melting points, specific

(1) Aided by grants from Stanley Drug Products, Inc., Division of Sperti Drug Corp., and the Lt. David Van Alstyne III Foundation.

(2) Presented in part at the 1st Central Regional Meeting of the American Chemical Society, Akron, Ohio, May 1968 (Abstracts, No. 21), and the 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, Abstract No. MEDI 50.

(3) (a) K. Tanaka, Y. Tsuchiya, N. J. Berberich, Jr., L. G. Nutini, and E. S. Cook, Abstracts of the 37th General Meeting of the Japanese Biochemical Society, Nagoya, Japan, Oct 1964; *Seikagaku*, **36**, 660 (1964); (b) K. Tanaka, Y. Tsuchiya, N. J. Berberich, Jr., A. J. Mukkada, L. G. Nutini, and E. S. Cook, *Appl. Microbiol.*, **16**, 1457 (1968); (c) A. J. Mukkada, L. G. Nutini, and E. S. Cook, *ibid.*, **18**, 641 (1969).

(4) Y. Tsuchiya, K. Tanaka, E. S. Cook, and L. G. Nutini, *ibid.*, **19**, 813 (1970).

(5) (a) M. Mangyo, *Seikagaku*, **36**, 735 (1964); (b) S. Okamoto and U. Okamoto, *Keio J. Med.*, **8**, 211 (1959); (c) S. Sherry, A. P. Fletcher, N. Alkjaersig, and W. D. Sawyer, *Trans. Ass. Amer. Physicians*, **72**, 62 (1959); (d) A. Alkjaersig, A. P. Fletcher, and S. Sherry, *J. Biol. Chem.*, **234**, 832 (1959); (e) A. Sjoerdsma and I. M. Milsson, *Proc. Soc. Exp. Biol. Med.*, **103**, 533 (1960).

(6) J. C. Sheehan and V. Frank, *J. Amer. Chem. Soc.*, **71**, 1856 (1949).

(7) R. A. Turner, *ibid.*, **75**, 2388 (1953).

(8) M. Bergmann and L. Zervas, *Chem. Ber.*, **65**, 1192 (1932).

(9) R. H. Sifferd and V. duVigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

(10) J. J. Pisano, J. D. Wilson, L. Cohen, D. Abraham, and S. Udenfriend, *ibid.*, **236**, 499 (1961).

TABLE I

ω-AMINOACYL-L-HISTIDINES AND RELATED COMPOUNDS

Compd	Method	Yield, %[a]	Mp, °C[b]	$[\alpha]^{25}D$, deg[c] (c 2, H_2O)	Formula[d]
6	Pht	30	173–174[e,f]	+17.6[f]	$C_8H_{12}N_4O_3$
7	Pht	25	251–252 dec[g]	+21.4[h]	$C_9H_{14}N_4O_3$
8	Pht	16	242–243 dec	+23.2	$C_{10}H_{16}N_4O_3$
	Z	84	242–244 dec[i]	+4.0[i]	$C_{10}H_{16}N_4O_3 \cdot H_2SO_4$
9[k]	Pht	21	238–239 dec	+23.2	$C_{11}H_{18}N_4O_3 \cdot H_2O$[l]
	Z	72	239–240 dec	+23.5	$C_{11}H_{18}N_4O_3 \cdot H_2O$
10[m]	Pht	40	236–237 dec	+25.0	$C_{12}H_{20}N_4O_3$
	Z	76	238–239 dec	+25.4	$C_{12}H_{20}N_4O_3$
HisOMe·2HCl		96	198–199[n]	+3.5	

[a] Based on histidine in **6**—**10**. [b] Melting points were determined by the capillary tube method and were uncorrected. [c] Specific optical rotations were taken with a Laurent polarimeter. [d] Analytical results for C, H, N were within ±0.4% of the theoretical values, except where indicated. [e] G. Losse and G. Müller [*Chem. Ber.*, **94**, 2768 (1961)] give mp 170–175°. Lit.[7] mp 175–176°. [f] M. Hunt and V. duVigneaud [*J. Biol. Chem.*, **127**, 43 (1939)] give mp 175° and $[\alpha]^{26}D$ +25° (*c* 1, H_2O) for $C_8H_{12}N_4O_3 \cdot HCl \cdot H_2O$. [g] Lit.[7] mp 253–256° dec; lit.[9] mp 260° dec; lit.[e] mp 260–262° dec. [h] Lit.[7] $[\alpha]^{22}D$ +21.7° (*c* 1.1, H_2O); lit.[9] $[\alpha]^{25}D$ +20.5° (*c* 2, H_2O); lit.[e] $[\alpha]^{20}D$ +21.9° (*c* 1, H_2O). [i] Lit.[10] mp 240° dec; lit.[f] mp 235° dec. [j] Lit.[f] $[\alpha]^{25}D$ +5° (*c* 1, H_2O). [k] Ir peaks (cm^{-1}) were 672, 726, 742, 752, 800. [l] C: calcd, 48.52; found, 49.05. N: calcd, 20.58; found, 20.10. [m] Ir peaks (cm^{-1}) were 666, 693, 747, 796, 815. [n] E. Fischer and L. H. Cone [*Justus Liebigs Ann. Chem.*, **363**, 107 (1908)] give mp 197–198; N. C. Davis [*J. Biol. Chem.*, **223**, 935 (1956)] gives mp 200–201; lit.[e] mp 199–200°.

TABLE II

R_f VALUES OF ω-AMINOACYL-L-HISTIDINES AND RELATED COMPOUNDS (R_f × 100)

	Tlc[a]					Pc[b]	
Compd	Solv 1	Solv 2	Solv 3	Solv 4	Solv 5	Solv 6	Solv 7
1	24	34	44	36	75	46	26
2	34	40	52	20	62	67	45
3	36	48	56	16	60	79	56
4	42	54	60	14	62	83	65
5	50	64	63	16	70	87	71
6	24	10	16	50	85	63	15
7	29	09	14	15	78	76	19
8	36	11	15	08	62	83	22
9	38	12	18	06	61	87	25
10	46	14	18	07	58	90	30
L-Histidine	42	18	16	49	84	66	22
L-Alanine	33	40	46	39	80	59	41

[a] Silica gel G (E. Merck) 250-μ plates, purchased from Analtech, Inc., were used for tlc. Solvent 1, PhOH–H_2O (75:25, w/v), pH 2.0; 2, *n*-BuOH–AcOH–H_2O (60:20:20), pH 2.4; 3, *i*-PrOH–formic acid–H_2O (77:4:19), pH 2.7; 4, *sec*-BuOH–MeCOEt–dicyclohexylamine–H_2O (55:15:10:20), pH 10.3; 5, $CHCl_3$–MeOH–17% NH_4OH (40:40:20), pH 11.6. [b] Whatman No. 1 papers were used for pc. Solvent 6, PhOH–H_2O (80:20, w/v), pH 1.6; 7, *n*-BuOH–AcOH–H_2O (60:15:25), pH 2.7.

optical rotations, and analytical data of the compounds are given in Table I. Table II gives the R_f values on tlc and paper chromatography (pc).

Testing Procedures.—*In vivo* antistaphylococcal activity was examined using female Swiss albino mice, 8–10 weeks old and weighing 20–25 g each. The challenge organism was a penicillin-resistant strain of *Staphylococcus aureus*, originally isolated from an infected tonsil, which has been maintained in our laboratories for years. It was preserved under refrigeration in the lyophilized state, and stock cultures were grown on Bacto Staphylococcus Medium 110 (Difco) slants once in every 6 months. The inoculum was prepared from 24-hr cultures on slants at 37°. The cells were centrifuged and washed twice with physiological saline (TC Tyrode Solution, Difco), then suspended in the same soln. Usually 0.5 ml of the suspension adjusted to give a 70% transmission on a nephelometer (2.6×10^8 organisms) caused 80–90% mortality in 4 days after subcutaneous challenge in the groin of mice. A total of 5 mg of each sample was given in equally divided doses sc 2 hr before and 4 hr after the injection of *S. aureus*.

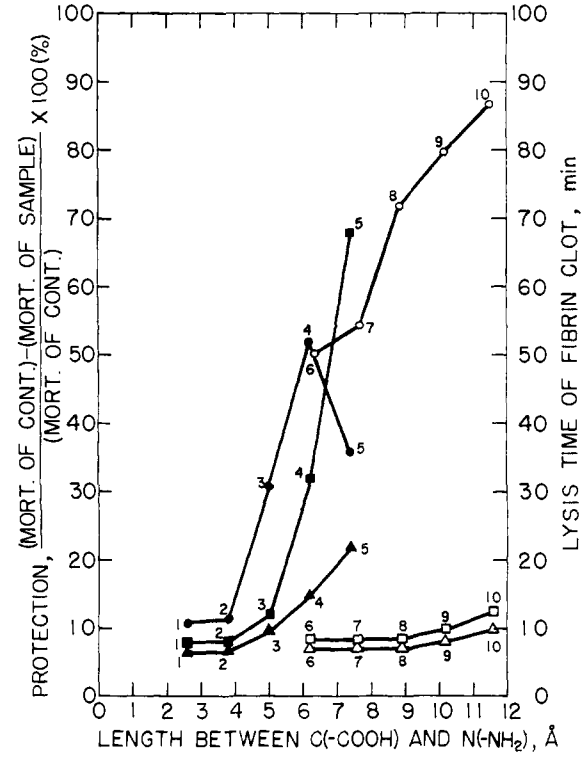


Figure 1.—Antistaphylococcal and antifibrinolytic activities of ω-amino acids and their L-histidine dipeptides. Numbers refer to compounds. The antistaphylococcal activity of each compound was established with 10 mice in each of 3 or more experiments: ω-amino acids (●—●), ω-aminoacyl-L-histidines (○—○). The antifibrinolytic activity was determined in 0.05 *M* phosphate buffer–saline solution with 10^{-5} *M* samples: ω-amino acids (■—■), ω-aminoacyl-L-histidines (□—□); and with 10^{-6} *M* samples: ω-amino acids (▲—▲), ω-aminoacyl-L-histidines (△—△).

Antifibrinolytic activity *in vitro* was determined by adopting the profibrinolysin–streptokinase system with expectation that these compounds would most likely affect the activation of profibrinolysin.[11] In a series of test tubes [8 (i.d.) × 74 mm] at 0° were mixed 0.1 ml of standard human serum (Microbiological Associates,

(11) S. Okamoto and U. Okamoto, *Keio J. Med.*, **11**, 105 (1962).

Inc.), 0.4 ml of sample dissolved in 0.05 *M* phosphate buffer saline, and 0.1 ml of saline containing 100 units of streptokinase (Varidase, Lederle Laboratories). Then 0.05 ml of saline containing 5 units of bovine thrombin (Mochida Pharmaceutical Co., Ltd.) and 0.3 ml of a 0.33% soln of bovine fibrinogen (Fraction I, Calbiochem) were added to the test tubes. The mixtures were incubated at 25° for 5 min. Then the test tubes were inclined at an angle of −20° to the horizontal and rapidly vibrated (70 vibrations/min, amplitude 15 mm)[12] to minimize the influence of the test tubes and other factors. The lysis time was measured by the number of minutes required for the clot to flow out from the test tubes.

Biological Results and Discussion.—The antistaphylococcal and antifibrinolytic activities of ω-amino acids and their histidine dipeptides are summarized in Figure 1. In a series of straight-chain ω-amino acids, **4** and **5** were demonstrated to provide better protection for mice against *S. aureus* than the smaller homologs, **3**, **2**, and **1**. All of the ω-aminoacyl-L-histidines were found to be more effective than their respective component ω-amino acids. Two newly synthesized histidine dipeptides, **9** and **10**, were shown to have greater antistaphylococcal activity than homocarnosine which previously was reported to be the most potent antistaphylococcal compound of this type.[3b]

The greatest antifibrinolytic activity for the ω-amino acids was found when the length of the CH_2 chain between the CO_2H and the NH_2 group was approximately 7 Å. This is almost the same pattern obtained in the antistaphylococcal activity. Further verification of this relationship was obtained with the very potent antifibrinolytic agent, *trans*-4-aminomethylcyclohexane-1-carboxylic acid, which at 5 mg produced 72% protection against staphylococcal infections (as effective as **8**). It was the most effective antifibrinolytic agent studied, as may be seen by comparing lysis times of 48 and > 180 min for 10^{-6} and 10^{-5} moles, respectively, with the values in Figure 1. From a consideration of the interrelationship between the antistaphylococcal and antifibrinolytic activities of ω-amino acids, it is possible that these ω-amino acids might inhibit the action of fibrinolysin which is activated by bacteriokinase, in this case staphylokinase. Thus, the protection of mice from staphylococcal infection by these ω-amino acids may be partially due to the antifibrinolytic action which may localize the bacterial infection, although direct observation under the microscope has not been attempted as yet.

However, all of the ω-aminoacyl-L-histidines were shown to have greater antistaphylococcal activity than their respective component ω-amino acids and yet no antifibrinolytic activity was obtained. The mechanism of the action of these dipeptides has not been established and must await further pharmacological studies. Possibly the ω-aminoacyl-L-histidines may stay longer in the mice, being incorporated into the tissues, and may gradually release the active ω-amino acids into the blood.

(12) The necessity of vibration was demonstrated by the following experimental results. With vibration fibrin clots in 83.3% of the control test tubes were dissolved in 7–9 min; without vibration, 85.0% were dissolved in 8–13 min.

Experimental Section

Phthalic anhydride was obtained from the Mallinckrodt Chemical Works. Glycine, Z-glycine, and Z-γ-aminobutyric acid were obtained from the Nutritional Biochemicals Corp. The ω-amino acids, **2**, **3**, **4**, **5**, and L-histidine, were purchased from Mann Research Laboratories, Inc. Z-β-Alanine and Z-ε-aminocaproic acid were obtained from the Sigma Chemical Co. Z-δ-Aminovaleric acid could not be obtained from commercial sources and was synthesized from **4**. L-Histidine methyl ester·2HCl was synthesized from L-histidine·2HCl. Solvents and other chemicals were obtained from Matheson Coleman and Bell. Melting points were taken by the capillary tube method and are uncorrected. Ir spectra (KBr) were taken with a Beckman infrared spectrophotometer, Model IR-20, at Matheson Coleman and Bell, Norwood, Ohio. Specific optical rotations were taken with a Laurent polarimeter. Elementary analyses were performed by the Crobaugh Laboratories, Cleveland, Ohio.

A. Phthalyl Method. Phthalyl-δ-aminovaleric Acid.—A stirred mixt of 12.0 g of phthalic anhydride and 9.0 g of **4** was heated at 150° for 30 min, cooled to room temp and crystd from hot H_2O: yield, 92.6%; mp 114–115°. *Anal.* ($C_{13}H_{13}NO_4$) C, H, N.

Phthalyl-δ-aminovaleryl Chloride.—A suspension of 16.4 g of Pht-δ-aminovaleric acid and 14.5 g of PCl_5 in 130 ml of C_6H_6 was heated in a water bath at 60° for 3 hr with stirring. The reaction mixt was coned *in vacuo* at 60° and the dry residue was crystd from C_6H_6 and pet ether (bp 40–49°): yield, 89.4%; mp 61–62°.

Phthalyl-δ-aminovaleryl-L-histidine.—To a soln of 7.9 g of L-histidine in 90 ml of H_2O and 20 ml of Me_2CO was added 2.0 ml of Et_3N, and the soln was cooled to approx −10° with the aid of a Dry Ice bath. A soln of 15 g of Pht-δ-aminovaleryl chloride in 60 ml of 1,4-dioxane was added slowly in 4 equal portions. The first portion was added to the histidine soln during a period of 30 min while the temp was maintained at −10°. Following the addition of 2.0 ml of Et_3N, the 2nd portion of the Pht-δ-aminovaleryl chloride soln was added in the same way. The 3rd and 4th portions were added in the same way as the 2nd portion, each being preceded by 2.0 ml of Et_3N. After the 4th addition 1.0 ml of Et_3N was added. The reaction mixt was allowed to warm to room temp with vigorous stirring and coned to dryness *in vacuo*. *n*-PrOH (40 ml) was added and the concn to dryness *in vacuo* was repeated. The residue was crystd from aq *n*-PrOH and recrystd from aq MeOH: yield, 26.0%; mp 220–222°; $[\alpha]^{25}D$ +15.0° (*c* 1, H_2O). *Anal.* ($C_{15}H_{20}N_4O_5$) H, N; C: calcd, 59.37; found, 58.58.

δ-Aminovaleryl-L-histidine (9).—A soln of 5.0 g of Pht-δ-aminovaleryl-L-histidine in 12 ml of H_2O was mixed with 3.0 ml of 5 *M* soln of hydrazine hydrate in EtOH. After 3 days the mixt of cryst Pht-hydrazine salt of δ-aminovaleryl-L-histidine and its soln was diluted with 25 ml of H_2O. Acidification with 1.0 ml of glacial AcOH caused pptn of Pht-hydrazine, which was then filtered off and washed well with cold H_2O. The filtrate was coned and purified by means of ion-exchange chromatography. For the separation on a column (1.8 × 30 cm) of Amberlite CG-120 resin, 0.1 *M* 2,6-lutidine buffer[13] was used. The PhOH–Ca(OCl_2) color reaction[14,15] was employed for the detection of the pure fractions of **4**, **9**, and histidine. Compounds **4** and **9** were identified by the clear blue color and histidine by a brown color. The color reaction was performed in the following manner: 0.5 ml of a sample from each fraction was mixed with 1.0 ml of 1% PhOH. The well-mixed soln, after addition of 0.1 g of $Ca(OCl)_2$ powder, was heated 5 min in a boiling water bath and then cooled in running H_2O. The peak quantity of **4** appeared in the effluent at approx 260 ml, histidine at 600 ml, and **9** at 1300 ml. The pure **9** fractions, 1100–1700 ml, were pooled and coned *in vacuo*. The dry residue was treated with H_2O–EtOH to produce crystals: yield, 89.0%; mp 238–239° dec; $[\alpha]^{25}D$ +23.2° (*c* 2, H_2O). *Anal.* ($C_{11}H_{18}N_4O_3 \cdot H_2O$) H; C: calcd, 48.52; found, 49.05. N: calcd, 20.58; found, 20.10.

(13) (a) D. Abraham, J. J. Pisano, and S. Udenfriend, *Arch. Biochem. Biophys.*, **99**, 210 (1962); (b) R. I. McManus, *J. Biol. Chem.*, **235**, 1398 (1960).

(14) J. W. Schick and E. F. Degering, *Ind. Eng. Chem.*, **39**, 906 (1947).

(15) (a) S. Kitaoka, K. Suzuki, and M. Itoh, Abstracts of the 40th General Meeting of the Japanese Biochemical Society, Osaka, Japan, Nov 1967, *Seikagaku*, **39**, 580 (1967); (b) S. Kitaoka and N. Nakano, Abstracts of the 41st General Meeting of the Japanese Biochemical Society, Tokyo, Japan, Oct 1968, *Seikagaku*, **40**, 554 (1968).

The acid hydrolysis (6 *N* HCl, 110°, 24 hr) of **9** produced **4** and histidine.

Phthalyl-ε-aminocaproic Acid.—The mixture of 14.8 g of phthalic anhydride and 13.1 g of **5** was allowed to react by the same procedure described for the synthesis of Pht-δ-aminovaleric acid: yield, 96.9%; mp 105–106°. *Anal.* ($C_{14}H_{15}NO_4$) C, H, N.

Phthalyl-ε-aminocaproyl chloride was prepd as described before: yield, 93.0%; mp 66–67°.

Phthalyl-ε-aminocaproyl-L-histidine was prepd as described before: yield, 46.0%; mp 225–226°; $[\alpha]^{25}D$ +16.6° (*c* 1, H_2O). *Anal.* ($C_{20}H_{22}N_4O_5$) H, N; C: calcd, 60.29; found, 57.37.

ε-Aminocaproyl-L-histidine (10) was prepd and purified as described before: yield, 86.0%; mp 236–237° dec; $[\alpha]^{25}D$ +25.0° (*c* 2, H_2O). *Anal.* ($C_{12}H_{20}N_4O_3$) C, H, N. The acid hydrolysis as described gave **5** and histidine.

B. Carbobenzoxy Method. Carbobenzoxy-δ-aminovaleric Acid.—To a soln of 20.6 g of **4** in 100 ml of 2 *N* NaOH in a flask cooled in an ice bath, 34 g of benzyl chloroformate and 50 ml of 4 *N* NaOH were added simultaneously to the vigorously stirred soln over a period of 20–25 min. The mixt was stirred for an additional 10 min. The soln was cooled in an ice bath and acidified to congo red with concd HCl. The ppt was filtered, washed with a small portion of cold H_2O, and dried in the desiccator *in vacuo*; yield, 60.4%; mp 102–103°. *Anal.* ($C_{13}H_{17}NO_4$) C, H, N.

L-Histidine Methyl Ester·2HCl.—A soln of 22.4 g of dry powdered L-histidine·2HCl and 320 ml of anhyd MeOH was heated on a water bath and dry HCl gas was introduced. The heating was continued for 3 hr. The resulting soln was cooled and allowed to stand in the ice bath and treated with Et_2O to furnish fine, colorless crystals which were recrystd from MeOH-Et_2O: yield, 96.0%; mp 198–199°; $[\alpha]^{25}D$ +3.5° (*c* 2, H_2O).

δ-Aminovaleryl-L-histidine (9).—To a soln of 12.5 g of Z-δ-aminovaleric acid in 250 ml of CH_2Cl_2 was added 7.0 ml of Et_3N. After the resulting soln had been chilled to −5°, 4.8 ml of ethylene chloroformate was added and the mixt was kept at the same temp for 10 min. To this soln was added rapidly a soln of L-histidine Me ester prepared by the addition of 21 ml of Et_3N to a soln of 12.2 g of L-histidine Me ester·2HCl in 250 ml of CH_2Cl_2 which had been chilled to 0°. The resulting mixt was stored at 25° for 2 days. It was then washed with 200 ml of H_2O and 200 ml of 1 *N* aq $NaHCO_3$, dried (Na_2SO_4), and concd to a syrup. It was dissolved in 100 ml of MeOH and 50 ml of 1 *N* aq NaOH was added. After storage for 3 hr at room temp, the soln was adjusted to pH 5 with 2 *N* H_2SO_4 and concd to dryness *in vacuo*. The syrupy residue was extd with two 50-ml portions of hot EtOH, and 50 ml of H_2O was added to the ext. After addition of 1.0 g of 10% Pd/C, the mixt was hydrogenated. The formation of CO_2 gas was checked occasionally until it ceased after 6 hr. The soln was filtered and concd *in vacuo*. The residual syrup was dissolved in 20 ml of H_2O and 2 *N* HCl added to give a pH below 5.0. The purification process by means of ion-exchange chromatography was the same as described before: yield, 71.8% (based on histidine Me ester·2HCl; mp 239–240° dec; $[\alpha]^{25}D$ +23.5° (*c* 2, H_2O). *Anal.* ($C_{11}H_{18}N_4O_3 \cdot H_2O$) C, H, N.

ε-Aminocaproyl-L-histidine (10).—Z-ε-Aminocaproic acid (13 g) was treated in the same way: yield, 76.0%; mp 238–239° dec; $[\alpha]^{25}D$ +25.4° (*c* 2, H_2O). *Anal.* ($C_{12}H_{20}N_4O_3$) C, H, N.

γ-Aminobutyryl-L-histidine (8).—Z-γ-Aminobutyric acid (12 g) was treated in the same way: yield, 83.6%; mp 242–244° dec; $[\alpha]^{25}D$ +4.0° (*c* 2, H_2O). *Anal.* ($C_{10}H_{16}N_4O_3 \cdot H_2SO_4$) C, H, N.

Acknowledgments.—We thank Dr. Leo G. Nutini, Professor of Biology and Experimental Medicine at the St. Thomas Institute, who established the anti-staphylococcal assay procedure, Dr. Floyd Green, of Matheson Coleman and Bell, for ir spectra, and Dr. Utako Okamoto, of the School of Medicine, Keio University, Tokyo, Japan, for valuable suggestions regarding the antifibrinolytic assay procedure. We are also indebted to the Ono Pharmaceutical Company, Ltd., Osaka, Japan, for a gift of γ-aminobutyryl-L-histidine monohydrate and the Daiichi Seiyaku Company, Ltd., Tokyo, Japan, for a gift of *trans*-4-aminomethylcyclohexane-1-carboxylic acid.

New Antiulcer Agents. 1. Syntheses and Biological Activities of 1-Acyl-2-, -3-, and -4-substituted Benzamidopiperidines

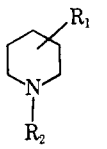
TSUTOMU IRIKURA* AND KAZUNORI KASUGA

Kyorin Chemical Laboratory, Kyorin Pharmaceutical Co., Ltd., Tokyo, Japan

Received August 17, 1970

A variety of 2-, 3-, and 4-substituted benzamidopiperidines was prepared from the corresponding benzamidopyridines by a Pd-catalyzed hydrogenation of the ring; syntheses of 1-acyl-2-, -3-, and -4-substituted benzamidopiperidines are reported. These compounds were tested for curative activity on the chronic gastric ulcer in rats using the clamping-cortisone method. Some of these compounds, particularly 1-(*p*-aminobenzoyl)-4-(3,4,5-trimethoxybenzamido)piperidine, showed some antiulcer activity. Structure–activity relationships are discussed.

Pharmacologically active piperidines having one substituent at the 2, 3, or 4 position of the ring are mainly of the following 3 types.



I. R_1
II. OR_1
III. NHR_1

Compounds I and II, where R_1 and R_2 signify various alkyl-, arylalkyl-, or CO-containing groups, have antihistaminic and antispasmodic activities. Aminopiperidine derivatives III, where R_1 and R_2 are various alkyl or arylalkyl groups, have been described as having antihistaminic and spasmolytic activities. However, diacyl compounds of type III do not appear to have been studied, although 1-benzoyl-3-benzamidopiperidine, 1-acetyl-4-acetamidopiperidine, and 1-benzoyl-4-benzamidopiperidine were obtained by Nienburg[1] and Tomita[2] in the course of the confirmation of the structure of aminopiperidine derivatives. We synthesized a series of 1-substituted benzoyl-2-, -3-, and -4-substituted benzamidopiperidines (IVa–IVc) and tested them for antiulcer activity by the clamping-cortisone method.

(1) H. Nienburg, *Chem. Ber.*, **70**, 635 (1937).
(2) K. Tomita, *Yakugaku Zasshi*, **71**, 1053 (1951).